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STUDIES IN EXPERIMENTAL ALCOHOLISM

BY

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STUDIES IN EXPERIMENTAL ALCOHOLISM.

By Reid Hunt.

Chief of the Division of Pharmacology, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

I.

THE EFFECT OF ALCOHOLISM UPON RESISTANCE TO ACETONITRILE.

Summary.—In the following experiments it is shown that animals to which alcohol has been administered for some time acquire an increased susceptibility to a definite poison (acetonitrile); this occurs after the administration of amounts of alcohol far too small to ever cause indications of intoxication and from doses which almost certainly cause no anatomical lesions which could be detected by present methods. It is shown that this increased susceptibility is not due to a general "lowering of resistance" but is associated with a distinctly increased power of the body to break up the molecule of acetonitrile; reasons are given for believing that this increased breaking up of the acetonitrile depends upon increased powers of oxidation on the part of the body.

It is believed that these experiments afford clear experimental evidence for the view that extremely moderate amounts of alcohol may cause distinct changes in certain physiological functions and that these changes may, under certain circumstances, be injurious to the body. The results also afford further evidence that in some respects the action of alcohol as a food is different from that of carbohydrates, and finally that in all probability certain physiological processes in "moderate drinkers" are distinctly different from those in abstainers.

The effects upon man of the moderate use of alcohol have long interested pathologists and physiologists as well as clinicians. The earlier efforts to solve this problem were made by pathological anatomists. After the anatomical changes resulting from the excessive use of alcohol had been recognized, pathologists turned their attention to the effects of smaller amounts of alcohol; for this purpose many experiments were made upon the lower animals, for it seemed probable that in this way better material for study could be obtained than from human subjects. As Professor Welch [a] points out, these anatomical studies on experimental alcoholism have, however, been distinctly disappointing and throw but little light on functional disturbances.

[a] Physiological Aspects of the Liquor Problem, Vol. II.

This is true notwithstanding the fact that animals were given intoxicating doses daily for years. Thus the experiments on swine of Dujardin-Beaumetz and Audigé (1879–1884) extending over several years were practically negative. In Friedenwald's experiments, carried out in Welch's laboratory, rabbits were given intoxicating doses of alcohol for long periods—sometimes for years; the results were stated by Welch to be meager. Doctor Welch further states, "No systematic experiments have been made to determine the pathological effects on animals of the long-continued use of alcohol in quantities so small as to produce no manifest symptoms of intoxication; but in view of the comparatively meager results in the experiments with moderate intoxicating doses, it seems improbable that experiments of the former character would yield positive results," and, further, that from the clinical side, however, "instances have been reported in increasing numbers in recent years of the occurrence of diseases of the circulatory, renal, and nervous systems, reasonably or positively attributed to the use of alcoholic liquors in persons who regarded themselves, or were regarded by others, as moderate drinkers." In many cases the injury was latent, and only manifested itself as the result of some accident or of an acute febrile disease. The relation of alcoholic liquors to gouty manifestations has long been recognized, as well as the increased liability of alcoholics to contract certain diseases or to contract them in especially severe form. Much has also been written, from both the clinical and experimental side, on the relation of alcohol to infection; the results, which are, as yet, not very concordant have recently been summarized by Meltzer.[a] In most, if not all, of this experimental work the alcohol was given in intoxicating doses.

In a series of experiments to be described in this paper I have found profound modifications of certain physiological processes to result in a comparatively short time from doses of alcohol so small that indications of intoxication never occurred. So far as I am aware this is the first series of experiments in which distinct physiological changes have been found to result from what may be called the strictly moderate use of alcohol. Although there may be some doubt as to the exact explanation of the results I have obtained, any positive results in this field may prove of interest.

Before describing these experiments, however, a few words may be said upon the more general effects of alcohol upon the metabolism. The views upon this subject and especially upon the effect of alcohol upon physiological oxidations have undergone great changes in the

[a] Brit. Med. Journ., Nov. 24, 1906, p. 1463; see also Meltzer, Amer. Med., vol. 4, p. 60; 1902, and Trommsdorff, Arch. f. Hyg., vol. 59, p. 1; 1906.

last few years, but it may be safely said that the older views are still generally current in medical circles and medical literature. Thus, the belief was formerly quite general that alcohol has a specific action in retarding the metabolism of body material, both fat and proteid; alcohol in moderate quantities was said to "prevent waste" or "conserve the tissues." Thus, the obesity so often found in alcoholics was attributed to a direct interference with the oxidation of fats; the increased excretion of uric acid, observed after alcohol, was attributed to diminished oxidation—the view then being held that urea was normally formed from uric acid and that the processes of oxidation involved were retarded by alcohol. "Later as the functions of the nonnitrogenous nutrients of food came to be better understood and the fact established that alcohol is oxidized, as they (the nonnitrogenous nutrients) are, in the body, became fully established, the view has become common that its effect in retarding or protecting metabolism is to be explained by its action as food rather than as a drug—that, in other words, it tends, by its own oxidation, to prevent the oxidation of other materials." [a] According to these newer views the obesity is due to an excess of food; i. e., the food remains unoxidized not because the body is rendered incapable of oxidizing it, but because an excess of more easily oxidizable food is provided.[b] The argument of diminished oxidation based on the increased excretion of uric acid is still supported, but in a form very different from the original. As this is about the only specific instance [c] in which such an action is attributed to alcohol a few words may be devoted to it. A brief review of the current views on the formation of uric acid will make this supposed relation clear. Uric acid is believed to arise from the nucleic acids of either the food or the tissue (and from hypoxanthin, of unknown origin, of the muscle), that having the former origin being the "exogenous," that of the latter, the "endogenous," uric acid. A specific intracellular enzyme—nuclease—hydrolyzes nucleic acids with the production of purin bases and other substances; the guanin and adenin thus formed are transformed under the influence of other specific enzymes—guanase and adenase—into xanthin and hypoxanthin, respectively; hypoxanthin is converted, by means of an oxidase, into xanthin and this, by

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[a] Atwater, Physiological Aspects of the Liquor Problem, Vol. II.

[b] Bunge attributes the effect of alcohol in causing obesity to its effect upon the brain which makes the person indisposed to muscular exercise (Leheb. der physiol. v. 2, p. 538, 1905.

[c] The experiments of Simonowsky and Schoumoff (Pflüger's Archiv, v. 33, p. 251; 1883–84) on the inhibiting action of alcohol upon the oxidation in the body of benzol to phenol are often quoted; apparently, however, no conclusions can be drawn from them on account of the inexactness of the method used for determining the phenol.

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further oxidation, into uric acid.[a] Thus, one factor in the amount of uric acid excreted is the extent to which these changes of hydrolysis and oxidation occur. The other factor is the extent to which the uric acid so formed is further oxidized, for many organs have been shown to contain an oxidase capable of destroying uric acid. Alcohol administered with purin-containing foods increases the output of uric acid in the urine[b] and the accepted explanation of this is that this increased output occurs because the alcohol inhibits the oxidation of the uric acid. This explanation probably accords best with the facts at present known, especially if certain of the views of Burian and Schur in regard to the "integral factor" be accepted. Still there are so many unknown factors in nucleic acid metabolism[c] that another explanation of the effect of alcohol does not seem to be entirely impossible, namely, that alcohol increases the activity of the enzymes (hydrolytic or oxidizing, or both) by which the uric acid is formed;[d] or it might be supposed to accelerate the formation, in muscle, of hypoxanthin which is subsequently converted into uric acid. Further, on the explanation that alcohol inhibits the oxidation of uric acid, it is difficult to see why it should not inhibit that of endogenous origin also, for it is not believed that all of that formed is normally excreted.[e]

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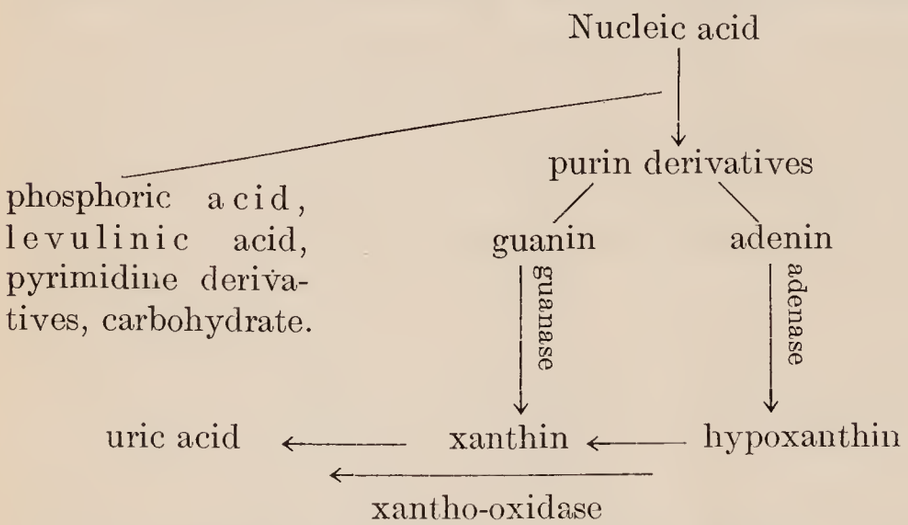
[a] These reactions are expressed by W. Jones in the following scheme:



[b] Beebe, Amer. Jour. Physiol., v. 12, p. 13; 1904. Cf. also Eschenburg (Münch. med. Woch., 1905, p. 2265), who obtained similar results with a patient suffering with gout; Rosenfeld, Einflüss des Alcohols auf den Organismus; Pringsheim, Zeit. für physikal. und diät. Therapie, v. 10, p. 284; 1906.

[c] Kossell and Steudel, for example, have suggested that certain pyrimidine bodies may be converted into uric acid.

[d] Burian (Hoppe-Seyler's Zeitschr., v. 43, p. 528; 1905) found tartronic, dialuric and salicylic acids to accelerate the conversion of purin bases into uric acid in extracts of the liver of the beef. Rockwood, at the December (1906) meeting of the American Chemical Society, reported experiments in which an increased output of endogenous uric acid was found to follow the administration of sodium salicylate.

[e] In any case the experiments quoted above are not conclusive as regards the effects of the habitual use of alcohol, for they were made upon those unaccustomed to the use of alcohol and were of but a few days' duration. In chronic alcoholism, Pollak (Dtsch. Arch. f. klin. Med., v. 88, p. 224; 1906) found a retention, or delayed excretion, of uric acid after the administration of nucleic acid.

In fact, both Rosenfeld and Pringsheim (who did not, however, take into consideration the difference in the effect of alcohol upon the endogenous and exogenous uric acid) attributed the increased excretion of uric acid, following the administration of alcohol, to an increased destruction of nucleic acid-containing proteids. In his striking way, Rosenfeld said that "alcohol hypocritically spared the ordinary proteids that it might rage all the more fiercely among the nucleoproteids without betraying itself in the nitrogen equilibrium."

This is mentioned simply to show that there seem to be no facts which necessarily point to alcohol, in small amounts, having an inhibiting effect upon physiological oxidations. In fact, as our knowledge concerning specific cases of oxidation in the body increases, it becomes more and more apparent that the commonly used expressions that a substance "accelerates" or "retards" physiological oxidation are far too general.

Thus there is more and more a tendency to discuss the effects of substances upon specific cases of oxidation, and I have been led to believe that alcohol probably causes increased oxidation in some cases. The substance with which I have chiefly experimented in this connection is acetonitrile. This substance may seem at first to have a purely theoretical interest, but from a long series of experiments with it I have been led to the conclusion that a thorough study of its conduct in the animal body may throw light upon a number of obscure processes of metabolism.[a] Chemically, acetonitrile (CH_3CN), or methyl cyanide, may be considered as hydrocyanic acid in which the hydrogen atom has been replaced by the methyl group. Both chemically and physiologically, however, acetonitrile is very different from hydrocyanic acid, although it is almost certain that its physiological action is due to the slow liberation of hydrocyanic acid in the body.

So far as I am aware acetonitrile has never been found in nature, although it has long been supposed that analogous compounds occur in living protoplasm, and importance has been attributed to them in certain disorders of metabolism.

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[a] The thyroid, for example, is quite generally supposed to have the power of effecting the neutralization of certain poisons of metabolic origin. These poisons are, however, purely hypothetical, and the only known poison toward which the thyroid has been shown to have an antidotal action is acetonitrile. In a previous paper (Journ. Biolog. Chem., vol. 1, p. 33; 1905) I have shown that animals to which small doses of thyroid have been administered for a short time will survive the injection of several times the fatal dose of acetonitrile. The explanation of this action is not known; if it were it would almost certainly throw much light on the physiology of the thyroid. It may also be noted in this connection that the feeding of parathyroids does not have such an effect; on the contrary, it slightly increases the susceptibility to acetonitrile. This affords another illustration of the fact that the thyroid and parathyroids have, in some respects at least, very different physiological effects.

Chemically the nitriles are chiefly characterized by their ability to unite with water, with the formation of corresponding organic acids and ammonia. In fact, this is one of the most usual methods of preparing many organic acids in the laboratory. Acetonitrile, for example, reacts with water in the presence of an alkali or acid, forming acetic acid and ammonia:

$$CH_3CN + 2H_2O = CH_3COOH + NH_3.$$

Giacosa, one of the earliest experimenters on this subject, thought that a similar reaction occurred in the animal body. He based this belief upon the fact that the urine of animals, poisoned with acetonitrile, gives a red color with ferric chloride. It was soon found, however, that the substance giving this color was not acetic acid, but sulphocyanic acid—HCNS.[a] It was also found that hydrocyanic acid, when administered to animals, reappeared in part in the urine as sulphocyanic acid. This observation led to the interesting experiments on the antidotal action of certain sulphur compounds toward hydrocyanic acid, and to the suggestion of a form of treatment of hydrocyanic acid poisoning, which would probably be the best yet proposed, if it could be given promptly enough.

The discovery of sulphocyanic acid in the urine after the administration of acetonitrile (and some other nitriles) was the beginning of our real pharmacological knowledge of acetonitrile. It showed that the changes undergone by this substance in the body are, in some respects at least, entirely different from those which take place in the test tube; *in vitro*, acetic acid is formed; in the organism hydrocyanic acid is produced, and this subsequently unites with sulphur to form sulphocyanic acid. The poisonous effects of acetonitrile are thus attributed to the slow formation of hydrocyanic acid. The methyl group of acetonitrile appears in the urine as formic acid. The process by which hydrocyanic acid is formed from acetonitrile does not seem to have been discussed by former writers. The most natural supposition would be that there is a simple hydrolysis of the acetoni-

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[a] The literature on the pharmacology of aceto- and other nitriles can be found by consulting the following:

Pelikan, Beiträge zur gericht. Med., Toxikol. und Pharmacodynamik, Würzburg, 1858, p. 93.

Giacosa, Zeitschr. f. physiol. Chem., v. 8, p.110; 1883–1884 .

Lang, Arch. exp. Path. und Pharmakol., v. 34, p. 247; 1894.

Heymans und Masoin, Arch. int. de Pharmacodynamie, v. 3, pp. 77, 359; v. 7, p. 297; v. 8, p. 1; Journ. of Physiol., v. 23, suppl. p. 23.

Verbrugge, Arch. int. de Pharmacodynamie, v. 5, p. 161; 1899.

Meurice, ibid., v. 7, p. 11; 1900.

Fiquet, ibid., v. 7, p. 307; 1900.

Hunt, ibid., v. 12, p. 447; 1904; Journal of Biolog. Chem., v. 1, p. 33, 1905.

Brissemort, C. R. Soc. Biol., v. 60, p. 54; 1906.

trile with the formation of methyl alcohol on the one hand and hydrocyanic acid on the other, according to the equation

$$CH_3CN + H_2O = CH_3OH + HCN.$$

Such a reaction has not been obtained by chemical processes outside of the body, and we have no reason to suppose that it can occur within the body.[a] A study of a number of related nitriles led me to form another conception of the process by which the hydrocyanic acid is formed. On comparing the toxicity of a number of nitriles I noticed that there is a relation between the degree of toxicity and the ease with which the hydrocarbon residue, to which the cyanogen group is united, is oxidized in the body. Thus acetonitrile is the least poisonous of the series of aliphatic nitriles studied; the methyl group is the most difficult of the hydrocarbon residues in these compounds for the body to oxidize. Proprionitrile (C_2H_5CN) is many times more poisonous than acetonitrile; the ethyl group (C_2H_5) is readily oxidized in the body. After the administration of acetonitrile, formic acid (a product of the oxidation of the methyl group) is found in the urine; this shows that the oxidation is incomplete; after proprionitrile, the urine contains no formic (or acetic) acid, the ethyl group having been completely oxidized. The difference in the ease of oxidation of the methyl and ethyl groups is most strikingly illustrated by the fate in the body of ethyl and methyl alcohol, respectively. Ethyl alcohol, even when administered in comparatively large doses to man or other animals, is completely oxidized within a few hours; methyl alcohol, on the other hand, even in small amounts, is imperfectly oxidized, and the process extends over days instead of hours—a fact which led pharmacologists[b] to utter a word of warning concerning the use of methyl as a substitute for ethyl alcohol years before there was a single well-known case of poisoning by it in man, a warning recently justified by the hundreds of cases of death and blindness caused by this substance.[c]

Having formed the conception that hydrocyanic acid is liberated from acetonitrile through the oxidation of the methyl group,[d] I began experiments to determine the effects upon the toxicity of the nitrile,

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[a] The formation of hydrocyanic acid from nitroprussiate of soda, on the other hand, is probably the result of a hydrolytic cleavage (Carquet, Thèse, Montpelier, 1903).

[b] See Hunt, Johns Hopkins Hospital Bulletin, XIII, p. 213; 1902.

[c] See Buller and Wood, Jour. Amer. Med. Assoc., Oct., 1904.

[d] An analogy to this supposed mode of decomposition is that of ammonium chloride in rabbits. Pohl and Münzer (Arch. f. exp. Path. u. Pharm., v. 43, p. 28) found that when this substance was administered to rabbits the ammonia was converted into urea while the hydrochloric acid was liberated and the animals died of acid intoxication; the fatal dose of the ammonium chloride corresponded closely to the equivalent amount of hydrochloric acid. In a similar manner Kohn and Czapek (Hofmeister's Beit., v. 8, p. 302; 1906) explain the injurious effects of certain salts upon the growth of fungi. Some fungi use up the kations and the organisms become poisoned by the acids formed from the anions; others use up the anions and become poisoned by the alkalies formed from the kations.

of various agents currently supposed to influence physiological oxidations. One of the first substances studied in this connection was alcohol. Although familiar with the view that alcohol diminishes oxidation, I was led to the hypothesis that oxidation in this case would be increased. This reasoning was based on a consideration of the probable cause of tolerance for alcohol. It is rather striking that so little attention has been paid to this factor in alcoholism. Although tolerance is one of the most familiar facts in connection with alcohol, I could find no reference to it in a bibliography on alcohol covering several hundred pages. One of the few drugs studied from the standpoint of tolerance is morphine. Faust [a] found that the establishment of tolerance for morphine was accompanied by an increased power on the part of the organism to destroy (oxidize) morphine, and he attributed the tolerance to this power.[b] It seemed reasonable to suppose that the tolerance for alcohol is accompanied by a similar increased power on the part of the body to oxidize alcohol and it was but a further step to suppose that, if the body became increasingly capable of oxidizing alcohol, it would also oxidize alkyl groups in general, such as the methyl group, more readily. If this should occur with the methyl group of acetonitrile, then animals accustomed to alcohol should be especially susceptible to this nitrile. Such was found to be the case. Animals which had received for a few weeks or months small amounts of alcohol—amounts far too small to ever cause any indications of intoxication—succumbed to doses of acetonitrile which produced no symptoms in the controls which had received no alcohol. These seem to be the first experiments in which marked functional disturbances have been found in animals which may be compared to strictly moderate drinkers. The first series of these experiments were performed upon mice.

EXPERIMENTS ON MICE.

As was pointed out in a recent paper [c] the susceptibility of mice to acetonitrile varies very considerably; the age of the animal, the character of the food, the temperature and season, all seem to have an influence. Hence in order to obtain satisfactory results it is necessary to have many controls. In the following experiments great care was taken to keep the conditions as uniform as possible, and a number of controls were made in every experiment.

The alcohol was administered by soaking the food (usually oats) in it. There was probably some loss by evaporation; but this was lessened by having the feeding cups narrow and deep. The strength of

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[a] Arch. f. exper. Path. u. Pharmakol., v. 44, p. 216; 1900.

[b] Hausmann (Pflüger's Archiv., 113, p. 337; 1906) has recently suggested that possibly the form in which the morphine is excreted changes as the tolerance is established, so that the drug can no longer be detected by the usual methods.

[c] Hunt, Journ. Biol. Chem., vol. 1, p. 33; 1905.

the alcohol was slowly increased from 5 or 10 per cent to 40 or 50 per cent. By carefully observing the weight of the mice and not increasing the strength of the alcohol too rapidly, it was possible to keep the animals for months on this diet without any material loss of weight. The acetonitrile was injected subcutaneously in aqueous solution.

White mouse.—Food consisted of oats covered with alcohol; the strength of the alcohol was gradually increased.

Date.	Weight of mouse.	Remarks.
	Grams.	
July 22, 1904	17	5 per cent alcohol.
July 31, 1904	.....	10 per cent alcohol
August 6, 1904	.....	15 per cent alcohol.
August 12, 1904	16.6	20 per cent alcohol.
August 19, 1904	16.7	25 per cent alcohol.
August 24, 1904	.....	30 per cent alcohol.
September 1, 1904	16.61	35 per cent alcohol.
November 3, 1904	17.36	3.99 milligrams acetonitrile (=0.23 milligram per gram) injected subcutaneously. Died in 2 hours.

Another mouse in this series died from 0.22 milligram per gram body weight; while another recovered from 0.18 milligram per gram. With a fourth one the alcohol was increased to 45 per cent; on December 24 it died from 0.25 milligram per gram. The weight of this mouse had increased during the alcohol period from 12 to 17.05 grams.

Control experiments to the above gave the following results: In one experiment the weight of the mouse had increased from 18.4 to 19.55 grams; it recovered from 0.36 milligram acetonitrile per gram. Another mouse, the weight of which had decreased from 18.05 to 17.72 grams, recovered from 0.38 milligram acetonitrile per gram body weight. A third one recovered from 0.39, a fourth one from 0.42, while a fifth one died from 0.4 milligram per gram.

Thus in this series the mice which had received alcohol died from about one-half the amount of acetonitrile as did those which had not received alcohol.

In another series the alcohol was increased more rapidly; the mice decreased a little in weight. Thus, for example:

Date.	Weight of mouse.	Remarks.
	Grams.	
May 17, 1904	21.51	20 per cent alcohol.
May 23, 1904	19.54	
June 27, 1904	20.15	
June 28, 1904	.....	30 per cent alcohol.
July 5, 1904	18.38	
July 6, 1904	.....	3.68 milligrams acetonitrile (=0.2 milligram per gram body weight). Died in 1 hour.

Other mice of this series died from 0.22 and 0.24, while one recovered from 0.18 milligram per gram body weight.

Control mice, namely, those which had been kept on water and oats, recovered from 0.4, 0.45, 0.5, milligram per gram, but one died from 0.55 milligram. Thus the alcohol mice succumbed to one-half to one-third the dose necessary to kill the controls.

Similar results were obtained in a number of other such experiments upon both white and gray mice. In no case did the mice show any symptoms of intoxication or any ill effects at all from the alcohol. There were only insignificant changes in weight, no greater than those in the controls, and the changes which did occur were as often in the direction of an increase as of a decrease.

The following experiments show in a striking manner how the susceptibility of mice may be increased by the feeding of alcohol. In the first experiment the mouse had been kept for four months in a small jar on a diet of oats soaked in water; its weight had remained practically unchanged throughout this period. At the end of this period acetonitrile, 0.5 milligram per gram body weight, was injected. The mouse recovered. It was then placed upon oats soaked in alcohol, the per cent of which was gradually increased to 45 per cent. After a little more than a month of this diet, 0.2 milligram acetonitrile per gram body weight proved fatal. The weight of the mouse had remained practically constant throughout this period. Control mice, which had received oats in water, recovered from 0.4 and 0.5 milligram per gram.[a]

In other experiments of this series, mice which had recovered from 0.4 and 0.5 milligram acetonitrile died, after six weeks of alcohol (during which they had maintained their body weight and had remained very active), from 0.23 and 0.27 milligram acetonitrile per gram body weight.

In another series mice which had been closely confined and kept on an oats diet for several months, and which had acquired a marked resistance to the poison, recovered from 0.7 to 0.8 milligram per gram; one after six weeks of the alcohol diet died from 0.4 milligram per gram, whereas the control (which had been kept on water) recovered from 0.8 milligram.

In other experiments of this character, in which similar results were obtained, the mice had lost some weight on the alcohol diet, and it was thought that this might have been a factor in their increased susceptibility. It will be shown below, however, that it is possible to reduce the weight of mice very considerably without causing any such increased susceptibility. In the following experiment the loss of weight was accompanied by a very marked increased resistance to the poison. In these experiments the mice were fed for some time upon a very

[a] It is very probable that these amounts were considerably less than the fatal dose, for it was found that mice kept in a small jar where they could obtain but little exercise, and on a diet of oats, became very tolerant for acetonitrile. This point will be referred to again.

limited amount of oats; there was a decrease in the body weight but a strikingly increased resistance to acetonitrile.

The following experiments will serve to illustrate these points:

Date.	Weight of mouse.	Remarks.
	Grams.	
January 18, 1905	18.21	Limited food begun.
January 24, 1905	17.01	
January 30, 1905	15.92	
February 6, 1905	16.42	
February 13, 1905	12.61	
February 15, 1905	14.11	On this morning the mouse was given full food, 16.93 milligrams acetonitrile (=1.2 milligrams per gram body weight). Survived.
February 16, 1905	.....	Alcohol, 5 per cent on oats.
February 21, 1905	.....	Alcohol increased to 10 per cent.
February 24, 1905	14.02	
February 27, 1905	.....	Alcohol increased to 20 per cent.
March 3, 1905	13.61	
March 10, 1905	14.10	Alcohol increased to 25 per cent.
March 18, 1905	14.05	
March 22, 1905	.....	Alcohol increased to 35 per cent.
March 29, 1905	14.25	Alcohol increased to 45 per cent.
April 5, 1905	15.32	
April 11, 1905	.....	Alcohol increased to 60 per cent.
April 14, 1905	14.85	4.46 milligrams acetonitrile (=0.3 milligrams per gram body weight). Died.

The following control experiments were made: (a) (To the first injection) other mice of the same lot, which had been allowed to eat all that they would, succumbed to 0.6 and 0.65 milligram per gram body weight, while others of the series, which had been kept on the reduced diet, survived 0.9 and 1 milligram acetonitrile per gram body weight; (b) (to the second injection) mice, which had been kept on oats and water for the same length of time that the others had received alcohol, survived 0.65 and 0.6 milligram per gram. Other experiments of this series are as follows:

Date.	Weight of mouse.	Remarks.
	Grams.	
February 16, 1905	23.16	Limited food begun.
February 21, 1905	18.85	
February 27, 1905	18.25	20.08 milligrams acetonitrile (=1.1 milligrams per gram body weight). Survived.
March 1, 1905	.....	Alcohol, 5 per cent on oats.
March 6, 1905	18.95	
March 7, 1905	.....	Alcohol increased to 10 per cent.
March 12, 1905	.....	Alcohol increased to 20 per cent.
March 14, 1905	19.61	
March 16, 1905	.....	Alcohol increased to 25 per cent.
March 21, 1905	19.26	Alcohol increased to 35 per cent.
March 29, 1905	18.92	Alcohol increased to 45 per cent.
April 5, 1905	19.05	
April 7, 1905	19.02	5.71 milligrams acetonitrile (=0.3 milligrams per gram). Died in 2 hours.

Controls (a) (that is, mice which had received full food as long as the above one had received limited food) died from 0.65 and 0.6 milligram per gram mouse; controls (b) (that is, mice which had received oats and water as long as the above had received oats and alcohol) survived 0.6 and 0.55 milligram.

Another striking experiment of this character is the following:

Date.	Weight of mouse.	Remarks.
	Grams.	
February 16, 1905	18.15	Limited food begun.
February 21, 1905	15.25	
February 28, 1905	13.57	27.14 milligrams acetonitrile (=2 milligrams per gram). Survived.
March 3, 1905	.....	5 per cent alcohol on food.
March 7, 1905	15.52	
March 8, 1905	.....	Alcohol increased to 10 per cent.
March 12, 1905	.....	Alcohol increased to 20 per cent.
March 14, 1905	17.85	
March 16, 1905	.....	Alcohol increased to 25 per cent.
March 21, 1905	18.27	Alcohol increased to 35 per cent.
March 29, 1905	18.80	Alcohol increased to 45 per cent.
April 5, 1905	19.35	
April 6, 1905	19.31	6.76 milligrams acetonitrile (=0.35 milligrams per gram). Died in 18 hours.

Thus this mouse, when receiving a limited amount of food, recovered from four times the absolute amount of nitrile which caused death after it had been given alcohol; it recovered from nearly six times the relative dose (that is, in proportion to body weight).

Two control experiments of this series are given in detail, for in them the mice, although receiving all the food they would eat, also lost some weight but did not show an increased resistance to the nitrile.

Date.	Weight of mouse.	Remarks.
	Grams.	
February 16, 1905	24.82	Full feed.
February 24, 1905	21.52	
February 27, 1905	21.57	14.02 milligrams acetonitrile (=0.65 milligrams per gram). Died in 4½ hours.

The mouse in the experiment described immediately before this one had recovered from 27.14 milligrams, or from nearly double the absolute amount which was fatal in this case; the relative figures (that is, in proportion to body weight) were 3 to 1.

In the following control a nonfatal dose of nitrile was given and then the usual diet (oats and water) continued.

Date.	Weight of mouse.	Remarks.
	Grams.	
February 16, 1905	20.46	Full food (oats and water).
February 24, 1905	18.12	
February 27, 1905	17.45	8.73 milligrams acetonitrile (0.5 milligrams per gram). Survived.
February 28, 1905	.....	Oats and water continued.
March 6, 1905	17.35	
March 14, 1905	17.82	
March 21, 1905	17.22	
March 29, 1905	17.75	
April 5, 1905	18.15	
April 7, 1905	17.82	12.98 milligrams acetonitrile (0.65 milligram per gram). Survived.

On comparing the second injection of the nitrile with that of the nitrile in the mouse on alcohol given above, p. 16, it will be seen that this mouse (on oats and water) recovered from 12.98 milligrams, whereas the mouse on alcohol died from 6.76 milligrams—the relative doses were 0.65 milligram and 0.35 milligram per gram body-weight. This experiment also illustrates what was invariably observed, namely, that one dose of nitrile did not render the mouse more susceptible to a second dose, and that a prolonged diet of oats and water tends to increase the resistance of mice to the poison. This experiment (and it is but one of many) also shows that the increased susceptibility of the alcohol mice which had received a previous injection of the nitrile can not be attributed to the latter.

In order to determine if poisons other than alcohol can, by "lowering the resistance," cause a similar increase in the susceptibility to acetonitrile, the following experiments were made:

Amyl alcohol.[a]—The oats, which formed the exclusive food of the mice, were covered with a solution, or emulsion, of amyl alcohol (Kahlbaum).

Date.	Weight of mouse.	Remarks.
	Grams.	
July 11, 1904	13.02	Amyl alcohol, 12 per cent.
July 18, 1904	12.05	
July 25, 1904	12.01	
August 5, 1904	10.70	
August 11, 1904	10.30	
August 15, 1904	10.96	3.84 milligrams acetonitrile (0.35 milligram per gram). Survived.

[a] This alcohol is not oxidized to any considerable extent in the body; the tolerance for it is probably not dependent upon oxidation processes; hence it seemed an especially favorable drug to compare with ethyl alcohol.

(Mice which had received ethyl alcohol up to 45 per cent for a similar period died from 0.2 and 0.23 milligram per gram.)

The mouse in the above experiment was placed upon oats soaked in ethyl alcohol.

Date.	Weight of mouse.	Remarks.
	Grams.	
August 17, 1904	.....	Ethyl alcohol, 5 per cent.
August 19, 1904	11.25	Alcohol increased to 15 per cent.
August 25, 1904	.....	Alcohol increased to 25 per cent
August 26, 1904	9.41	
August 31, 1904	9.85	
September 14, 1904	10.40	
September 21, 1904	11.43	
September 28, 1904	11.30	
October 5, 1904	11.86	
October 12, 1904	11.50	
October 20, 1904	12.80	
October 28, 1904	12.77	Alcohol increased to 35 per cent.
November 4, 1904	14.53	2.91 milligrams acetonitrile (0.2 milligram per gram). Died in 9 hours.

In the following experiment the strength of the amyl alcohol was increased gradually:

Date.	Weight of mouse.	Remarks.
	Grams.	
May 23, 1904	23.07	Amyl alcohol, 2 per cent.
May 25, 1904	.....	Amyl alcohol increased to 4 per cent.
May 28, 1904	.....	Amyl alcohol increased to 6 per cent.
May 31, 1904	23.80	
June 4, 1904	.....	Amyl alcohol increased to 8 per cent.
June 6, 1904	23.63	
June 10, 1904	.....	Amyl alcohol increased to 10 per cent.
June 13, 1904	22.58	
June 20, 1904	22.05	
June 21, 1904	.....	Amyl alcohol increased to 12 per cent.
June 27, 1904	21.05	
July 5, 1904	20.98	
July 7, 1904	20.98	11.64 milligram acetonitrile (0.55 milligram per gram.) Survived.

Control mice which had been given ethyl alcohol died from 0.35 and 0.3 milligrams acetonitrile per gram.

Hydrated chloral.—Experiments similar to the above were performed with hydrated chloral. Although this drug frequently caused a considerable loss of weight, no increased susceptibility to acetonitrile was observed—thus:

Date.	Weight of mouse.	Remarks.
	Grams.	
January 9, 1905	17.92	Oats soaked in a 5 per cent solution of hydrated chloral.
January 13, 1905	15.45	
January 16, 1905	14.13	
January 22, 1905	13.71	4.1 milligrams acetonitrile (0.3 milligram per gram). Survived.
July 18, 1904	24.38	Oats in 5 per cent solution of hydrated chloral.
July 26, 1904	22.41	
August 5, 1904	20.62	
August 9, 1904	20.96	6.29 milligrams acetonitrile (0.3 milligram per gram). Survived.

(Control mice on alcohol, increased up to 45 per cent, died from 0.22 milligram per gram.)

The latter mouse was now placed upon ethyl alcohol:

Date.	Weight of mouse.	Remarks.
	Grams.	
August 10, 1904	.....	5 per cent alcohol in oats.
August 15, 1904	21.11	Alcohol increased to 15 per cent.
August 25, 1904	.....	Alcohol increased to 25 per cent.
August 26, 1904	20.70	
August 31, 1904	20.92	Alcohol increased to 35 per cent.
September 14, 1904	18.05	
October 20, 1904	19.20	
November 2, 1904	19.56	4.3 milligrams acetonitrile (0.22 milligram per gram). Died in 3 hours 50 minutes.

In the following experiments the difference between the effect of alcohol and hydrated chloral is still more marked.

Date.	Weight of mouse.	Remarks.
	Grams.	
May 31, 1904	19.82	Oats in 25 per cent alcohol.
June 6, 1904	19.72	
June 13, 1904	18.32	
June 20, 1904	19.55	
June 27, 1904	18.54	
July 5, 1904	19.71	
July 7 1904	19.71	2.95 milligrams acetonitrile (0.15 milligram per gram). Died in 3 hours.

Another mouse of the same lot was placed upon oats soaked in a 5 per cent solution of hydrated chloral.

Date.	Weight of mouse.	Remarks.
	Grams.	
May 31, 1904	16.38	Oats in 5 per cent hydrated chloral.
June 6, 1904	15.56	
June 13, 1904	15.37	
June 20, 1904	14.60	
June 27, 1904	13.73	
July 5, 1904	13.50	
July 6, 1904	13.50	4.73 milligrams acetonitrile (0.35 milligram per gram). Survived.

Another mouse of this series whose weight on the chloral diet had decreased from 26.6 to 19.27 grams also survived the injection of 0.35 milligram per gram.

Methyl alcohol.—A few experiments were performed in which the food was soaked in methyl alcohol. This alcohol, when continued for a short time, proved to be far more poisonous to mice than did ethyl alcohol, as has been shown to be the case for other animals;[a] no distinct increased susceptibility for acetonitrile was, however, noted.

Hydrochloric acid, etc.—A number of experiments were performed in which the food of the mice was soaked in 0.1 to 0.5 per cent hydrochloric acid. There was usually a marked loss of weight but no increased susceptibility to acetonitrile; on the contrary the resistance seemed to be increased. The feeding of potassium iodide, in doses sufficient to cause a marked loss of weight, increased, rather than decreased, the resistance to acetonitrile. A very large number of other substances (such as chloroform water, saccharine, sulphonal, thymol, tobacco, sodium benzoate and salicylate) were tried, but with negative results; only certain proteid substances gave results similar to alcohol.

Experiments with other cyanogen compounds.—A number of experiments were performed in which hydrocyanic acid and nitroprussiate of soda, sodium sulphocyanate, etc., were injected into mice which had been given alcohol for some time, the object being to see if the alcohol had caused a general "lowering of resistance" which would cause them to succumb to a smaller dose than the one fatal to normal mice. The results were negative; the alcohol mice were not more susceptible to these poisons than were the normal ones. The following experiments with nitroprussiate of soda illustrate this point:

Date.	Weight of mouse.	Remarks.
	Grams.	
July 25, 1904	14.98	5 per cent alcohol on oats.
July 31, 1904	.....	Alcohol increased to 10 per cent.
August 6, 1904	.....	Alcohol increased to 15 per cent.
August 8, 1904	14.51	
August 13, 1904	.....	Alcohol increased to 20 per cent.
August 15, 1904	15.22	
August 16, 1904	.....	Alcohol increased to 25 per cent.
August 19, 1904	15.70	
August 24, 1904	.....	Alcohol increased to 30 per cent.
August 31, 1904	16.40	
September 1, 1904	.....	Alcohol increased to 35 per cent.
October 5, 1904	15.95	
November 3, 1904	16.77	0.168 milligram nitroprussiate of soda (0.01 milligram per gram). Survived.

[a] See Hunt: The toxicity of methyl alcohol, The Johns Hopkins Hospital Bulletin, p. 213; 1902.

Another mouse of this series also recovered from 0.01 milligram per gram body weight of nitroprussiate of soda. Of the control mice which had received oats soaked in water, one died from 0.015 milligram of nitroprussiate of soda, another from 0.01, while a third recovered from 0.008 milligram per gram. Mice which had received the same amount of alcohol for the same period showed an increased susceptibility to acetonitrile.

Results similar to the above were obtained with hydrocyanic acid, sodium sulphocyanate, and guanidin carbonate.

The above results show that mice to which alcohol has been administered for some time are distinctly more susceptible to acetonitrile than are those which have received no alcohol; also that there is apparently something special about the poisonous action of alcohol, for certain other poisons which cause a loss of weight and so might be considered as agents which would probably "lower the general resistance" do not have this effect, and finally that the increased susceptibility which the alcohol mice show toward acetonitrile seems to be a special case for such mice as do not show an increased susceptibility toward other poisons related to acetonitrile (hydrocyanic acid, nitroprussiate of soda, and sodium sulphocyanate).

It was pointed out above (p. 10) that the formation of sulphocyanate from acetonitrile seemed to be a protective reaction on the part of the organism, the sulphocyanate being less poisonous than the hydrocyanic acid formed from the nitrile. Hence it might be supposed that the increased susceptibility of the alcohol mice to acetonitrile is due to a diminution of the power of the body to convert the liberated hydrocyanic acid into the sulphocyanate; in this case there should be a smaller excretion of sulphocyanate after nitrile in the animals on alcohol than in the normal. On the other hand, the hypothesis which led to these experiments was that as the body, as the result of the repeated administration of alcohol, acquired the power of oxidizing more and more of the hydrocarbon residues of alcohol, it also acquired the power of oxidizing more and more of the methyl group of the nitrile, by which process more and more of the cyanogen would be set free. The cyanogen thus formed might or might not combine with sulphur to form sulphocyanate. If the former occurred, the lack of an increased excretion of sulphocyanate in the urine would not necessarily mean that there had not been increased decomposition of the nitrile. On the other hand, if the alcohol animal excreted an increased amount of sulphocyanate, this would be strong evidence that there had been increased destruction of the nitrile with an increased formation of cyanogen and the increased toxicity could thus be explained.

In order to test the above hypotheses, determinations of the sulphocyanate in the urine were made after the administration of acetonitrile

to both normal and alcoholic animals. These experiments were performed for the most part upon guinea pigs for reasons given below.[a]

Methods.—In the earlier experiments, the sulphocyanate was extracted from the acidified urine with ether and determined, approximately, colorimetrically; later it was extracted with ether and determined volumetrically. Neither method gave satisfactory results, so that in subsequent experiments Lang's volumetric method was employed—titrating the urine with silver nitrate (this gives chlorine and sulphocyanate) and deducting from this the chlorine, determined by incinerating another portion of the urine with sodium carbonate and potassium nitrate. This method gave entirely satisfactory results with the urine of guinea pigs and usually with that of dogs. There seemed to be nothing present in the guinea pigs' urine which was precipitated by the silver nitrate except the chlorine and sulphocyanate. The method was not as a rule satisfactory in the case of the urine of the cat or rabbit, as the urine of these animals usually contained substances which, like chlorine and sulphocyanate, are precipitated by silver nitrate; the same was found to be the case with a few dogs.

EXPERIMENTS ON GUINEA PIGS.

Attention was called above to the variations in the susceptibility of normal mice to acetonitrile; some of the conditions influencing this are the age of the animals, the character of the food, the season, etc. All of these factors seem to be equally important in the case of the guinea pig; they also seem to influence the amount of acetonitrile which is converted into sulphocyanate. The per cent of acetonitrile which is converted into sulphocyanate seems also in part to depend upon the dose.

In the following experiments, care was taken to keep the conditions uniform; guinea pigs of the same age and weight were selected and they were then kept under identical conditions, except that to the food of some alcohol instead of water was added. The experiments were performed upon the same day, and in all cases duplicate analyses were made. The urine was collected and analyzed as long as it gave a decided reaction for sulphocyanate. The urine of the guinea pig, both of the normal and of those receiving alcohol, was found to be practically free of sulphocyanate; in some cases a faint qualitative test was obtained, but the amount was too small to determine.

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[a] These experiments were begun with Mr. E. S. Clowes, formerly assistant in the laboratory, and to whom I wish to express my thanks for his valuable assistance. It was our intention to extend our studies to other animals and to other conditions, but other work and Mr. Clowes's resignation have prevented this.

The following experiments show that in every case the animal which had received alcohol excreted a larger amount of sulphocyanate after corresponding amounts of the nitrile than did the normal. The guinea pigs on alcohol showed no ill effects whatever from the alcohol; they grew as rapidly and had as many young as the controls.

Date.	Weight of guinea pig.	Remarks.
	Grams.	
October 4, 1905	407	The food consisted largely of oats and bran soaked in 10 per cent alcohol; on about every third day a little green food was given.
October 10, 1905	430	Alcohol increased to 20 per cent.
October 20, 1905	470	Alcohol increased to 30 per cent.
November 2, 1905	467	
November 8, 1905	475	
November 14, 1905	460	
November 15, 1905	465	93 milligrams acetonitrile (0.2 milligram per gram weight). Survived.

On the first three days after the administration of the nitrile the following amounts of cyanogen were found in the urine:

Day.	Cyanogen.
	Milligrams.
First day	8.2
Second day	1.5
Third day	41.7
Total	51.4

Ninety-three milligrams acetonitrile (the amount injected) contains 58.96 milligrams cyanogen; hence about 87.2 per cent of the cyanogen of the nitrile appeared in the urine as sulphocyanate.

The control experiment was as follows:

Date.	Weight of guinea pig.	Remarks.
	Grams.	
October 4, 1905	410	Food as in preceding except that water was substituted for the alcohol.
October 10, 1905	425	
October 20, 1905	485	
November 8, 1905	475	
November 14, 1905	485	
November 15, 1905	490	98 milligrams acetonitrile (0.2 milligram per gram). Survived.

The excretion of cyanogen was as follows:

Day.	Cyanogen.
	Milligrams.
First day	6.2
Second day	23.8
Third day	1.5
Total	31.5

Ninety-eight milligrams acetonitrile (the amount injected) contains 62.13 milligrams cyanogen; hence 50.7 per cent of the cyanogen of the nitrile appeared in the urine as sulphocyanate.

Comparing this result with that of the alcohol experiment it will be seen that in the latter the excretion of sulphocyanate was 1.7 times as great as in the normal.

In the following experiment there were two controls; the administration of alcohol was continued for a longer period. The guinea pig on alcohol had been receiving dilute alcohol on its food from its birth eight months previously.

Date.	Weight of guinea pig.	Remarks.
	Grams.	
November 22, 1905	380	30 per cent alcohol on part of food.
November 29, 1905	365	
December 4, 1905	390	
December 9, 1905	405	
December 14, 1905	405	
December 21, 1905	431	
December 29, 1905	425	
January 2, 1906	.....	Alcohol increased to 40 per cent.
January 5, 1906	450	
January 12, 1906	480	
January 16, 1906	.....	Alcohol increased to 50 per cent.
January 19, 1906	520	
January 26, 1906	520	
February 2, 1906	540	
February 6, 1906	520	104 milligrams acetonitrile (0.2 milligram per gram). Survived.

The excretion of cyanogen was as follows:

Day.	Cyanogen.
	Milligrams.
First day	0.39
Second day	20.93
Third day	3.51
Fourth day	3.38
Fifth day	4.81
Sixth day	4.16
Total	37.18

One hundred and four milligrams acetonitrile contains 65.94 milligrams cyanogen; hence 56.3 per cent of the cyanogen of the nitrile was excreted as sulphocyanate.

The controls (of the same litter as the above) gave the following results:

Date.	Weight of guinea pig.	Remarks.
	Grams.	
November 22, 1905	330	Food as in foregoing, except for the alcohol.
December 4, 1905	375	
December 9, 1905	400	
December 14, 1905	405	
December 21, 1905	445	
December 29, 1905	435	
January 5, 1906	445	
January 12, 1906	475	
January 19, 1906	490	
January 26, 1906	515	
February 2, 1906	520	
February 6, 1906	500	100 milligrams acetonitrile (0.2 milligram per gram). Survived.

The excretion of cyanogen was as follows:

Day.	Cyanogen.
	Milligrams.
First day	4.03
Second day	.65
Third day	1.56
Fourth day	8.97
Fifth day	3.51
Sixth day	3.64
Total	22.36

One hundred milligrams acetonitrile (the amount injected) contains 63.4 milligrams cyanogen; hence about 35.2 per cent of the cyanogen of the nitrile appeared in the urine as sulphocyanate.

Comparing this result with that of the above alcohol experiment, it will be seen that the guinea pig on alcohol excreted about 1.65 times as much cyanogen as sulphocyanate as did the normal.

The other control experiment gave the following results:

Date.	Weight of guinea pig.	Remarks.
	Grams.	
November 22, 1905	295	
December 4, 1905	325	
December 9, 1905	355	
December 14, 1905	380	
December 21, 1905	425	
December 29, 1905	400	
January 5, 1906	390	
January 12, 1906	340	Had two young about this time.
January 19, 1906	345	
January 26, 1906	375	
February 2, 1906	400	
February 6, 1906	355	71 milligrams acetonitrile (0.2 milligram per gram). Survived.

The cyanogen excreted was as follows:

Day.	Cyanogen.
	Milligrams.
First day	3.38
Second day	2.99
Third day	3.51
Fourth day	1.17
Fifth day	1.56
Sixth day	2.34
Total	14.95

Seventy-one milligrams acetonitrile contained 45 milligrams cyanogen; hence 33.2 per cent of the cyanogen of the nitrile had appeared in the urine as sulphocyanate. Thus the guinea pig on alcohol had excreted 1.75 times as much cyanogen as sulphocyanate as did the normal after corresponding doses of the nitrile.

Another guinea pig of this series, which had received alcohol for several months (during which time its weight had increased from 345 to 520 grams), died from 95 milligrams acetonitrile (0.2 milligram per gram). And it may be remarked that a few other experiments were lost by the death of guinea pig receiving alcohol, for alcohol increased the susceptibility of guinea pigs to acetonitrile in the same way it did that of mice.

In the following experiments the excretion of cyanogen was less than in the foregoing, but a similar difference between the normal and alcohol animals was observed:

Date.	Weight of guinea pig.	Remarks.
	Grams.	
October 4, 1905	400	10 per cent alcohol on part of food.
October 10, 1905	420	
October 14, 1905	.....	Alcohol increased to 20 per cent.
October 20, 1905	440	
October 24, 1905	.....	Alcohol increased to 30 per cent.
November 2, 1905	435	
November 8, 1905	450	
November 14, 1905	460	
November 21, 1905	500	
November 28, 1905	490	Alcohol increased to 40 per cent.
December 3, 1905	530	106 milligrams acetonitrile (0.2 milligram per gram). Survived.

The excretion of cyanogen was as follows:

Day.	Cyanogen.
	Milligrams.
First day	10.5
Second day	7.4
Third day	5.5
Fourth day	4.8
Total	28.2

One hundred and six milligrams acetonitrile contains 67.2 milligrams cyanogen; hence 41.9 per cent had been excreted as sulphocyanate.

The control experiment was as follows:

Date.	Weight of guinea pig.	Remarks.
	Grams.	
October 4, 1905	385	Food as in the above, except for the alcohol.
October 10, 1905	410	
October 20, 1905	450	
November 8, 1905	450	
November 14, 1905	435	
December 3, 1905	505	101 milligrams acetonitrile (0.2 milligram per gram). Survived.

The cyanogen excretion was as follows:

Day.	Cyanogen.
	Milligrams.
First day	2.7
Second day	5.2
Third day	4.0
Fourth day	2.7
Total	14.6

One hundred and one milligrams acetonitrile contains 64 milligrams cyanogen; hence but 22.8 per cent of the cyanogen was excreted as sulphocyanate. The guinea pig on alcohol had converted 1.84 times as much of the cyanogen of the nitrile into sulphocyanate as the normal.

Thus in all of the above experiments the guinea pig which had been receiving alcohol excreted much more sulphocyanate after the administration of acetonitrile than did the normal; in most cases this excretion was about 1.7 times as great. The same results were obtained in a number of other experiments, the only exceptions being in a few experiments in which the alcohol guinea pig became very sick after the nitrile; in such cases there was either no greater excretion of sulphocyanate or there was a smaller excretion; but in all these experiments the guinea pig finally died, although death was sometimes delayed for a week.

The increased formation of sulphocyanate found in the above experiments with alcohol admits of two possible explanations: (1) there may have been an increased formation of sulphocyanate due to an increased breaking up of the acetonitrile molecule; or (2) equal amounts of sulphocyanate may have been formed, but normal animals may have the power of destroying more sulphocyanate. The latter supposition would be more in accord with the rather generally held view that oxidation processes are lowered by alcohol.

On the other hand, recent experiments by Pollak indicate that normal animals (dog, rabbit, man) destroy no sulphocyanate when this is administered as such.[a] My experiments on guinea pigs are in accord with Pollak's results with other animals in showing that there is no great destruction of sulphocyanate when given subcutaneously; and no difference was observed between normal and alcohol animals in this respect.

Fate of sulphocyanate in normal and alcohol guinea pigs.—My experiments with the subcutaneous injection of sodium sulphocyanate into guinea pigs were not very satisfactory, for although death did not result immediately there was usually much necrosis about the point of injection and death frequently occurred several days later.

Examples of such experiments are as follows: The first experiment was upon one of the guinea pigs which had been used for a nitrile experiment (see last experiment above); the alcohol had been

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[a] Hofmeister's Beit., v. 2, p. 430. A similar result had been obtained by Salkowski (Virchow's Archiv., v. 58, p. 460; 1873); Bruylants and Lang believed that much sulphocyanate was destroyed in the organism. The results of Pollak as regards the administration of sulphocyanate *per os* to man are in direct opposition to those of Edinger and Clemens (Zeit. für klin. Med. v. 59, p. 218); these authors found less than half of the sulphocyanate to be excreted.

continued from December 3. There had been an increase in weight, as shown below.

Date.	Weight of guinea pig.	Remarks.
	Grams.	
December 14, 1905	485	
December 21, 1905	570	
December 29, 1905	540	
January 2, 1906	568	170.4 milligrams sodium sulphocyanate (0.3 milligram per gram) subcutaneously. Died in 3 days, 14 hrs.; considerable necrosis about point of injection.

The excretion of cyanogen was as follows:

Date.	Cyanogen.
	Milligrams.
First day	24.8
Second day	6.7
Third day	6.4
Total	37.9

Thirty-seven and nine-tenths milligrams cyanogen corresponds to 118 milligrams sodium sulphocyanate; hence 69.3 per cent of the sodium sulphocyanate appeared in the urine.

The control (see above for early history) was as follows:

Date.	Weight of guinea pig.	Remarks.
	Grams.	
December 14, 1905	540	
December 21, 1905	600	
December 29, 1905	500	
January 2, 1906	602	180.6 milligrams sodium sulphocyanate (0.3 milligram per gram) subcutaneously. Recovered, but there was considerable necrosis about point of injection.

The excretion of cyanogen was as follows:

Day.	Cyanogen.
	Milligrams.
First day	33.8
Second day	8.3
Third day	0.0
Total	42.1

Forty-two and one-tenth milligrams cyanogen corresponds to 131.2 milligrams sodium sulphocyanate; hence 72.6 per cent of the sodium sulphocyanate injected had reappeared in the urine.

In the following experiments smaller doses of the sulphocyanate were given; there was but little necrosis.

Date.	Weight of guinea pig.	Remarks.
	Grams.	
December 11, 1905	200	Part of food soaked in 50 per cent alcohol.
December 18, 1905	205	
January 2, 1906	270	
January 8, 1906	325	
January 18, 1906	375	
January 25, 1906	400	
February 15, 1906	485	
February 28, 1906	545	
November 6, 1906	670	
November 11, 1906	630	126 milligrams sodium sulphocyanate (0.2 milligram per gram) subcutaneously. Survived.

The excretion of cyanogen was as follows:

Day.	Cyanogen.
	Milligrams.
First day	30.30
Second day	5.35
Third day	3.25
Total	38.90

Thirty-eight and nine-tenths milligrams cyanogen corresponds to 121.2 milligrams sodium sulphocyanate; hence 96 per cent of the sodium sulphocyanate was recovered from the urine.

The control was as follows:

Date.	Weight of guinea pig.	Remarks.
	Grams.	
December 13, 1905	150	Food as in the preceding experiment except for the alcohol.
January 2, 1906	225	
January 18, 1906	300	
February 15, 1906	385	
February 28, 1906	430	
March 23, 1906	490	
July 16, 1906	587	
October 6, 1906	670	
October 11, 1906	700	140 milligrams sodium sulphocyanate (0.2 milligram per gram) subcutaneously. Survived.

The excretion of cyanogen was as follows:

Day.	Cyanogen.
	Milligrams.
First day	32.35
Second day	2.60
Third day	4.64
Total	39.59

Thirty-nine and fifty-nine hundredths milligrams cyanogen corresponds to 123.4 milligrams sodium sulphocyanate; 88 per cent of the sodium sulphocyanate administered was therefore recovered from the urine.

In the following experiments the sodium sulphocyanate was administered *per os;* this method is unsatisfactory since the element of absorption from the intestinal tract is involved.

Date.	Weight of guinea pig.	Remarks.
	Grams.	
December 13, 1905	505	30 per cent alcohol on part of food.
December 29, 1905	435	
January 2, 1906	.....	40 per cent alcohol on part of food.
February 2, 1906	510	
February 6, 1906	.....	50 per cent alcohol on part of food.
March 5, 1906	450	
April 13, 1906	690	
June 22, 1906	620	
July 13, 1906	730	
September 17, 1906	710	213 milligrams sodium sulphocyanate (0.3 milligram per gram) *per os.* Survived.

The excretion of sulphocyanate (calculated as the sodium salt) was as follows:

Day.	Sodium sulphocyanate.
	Milligrams.
First day	75.33
Second day	27.14
Third day	8.51
Total	110.98

Thus 52.1 per cent of the sodium sulphocyanate administered *per os* reappeared in the urine in three days.

The control experiment was as follows: The guinea pig belonged to the same litter as the above and had been kept on the same food (with the exception of the alcohol) since December, 1905.

Date.	Weight of guinea pig.	Remarks.
	Grams.	
September 17, 1906	670	201 milligrams sodium sulphocyanate (0.3 milligram per gram) *per os*.

The secretion of sulphocyanate (calculated as the sodium salt) was as follows:

Day.	Sodium sulphocyanate.
	Milligrams.
First day	99.23
Second day	40.10
Third day	5.27
Total	144.60

In this case 71.9 per cent of the sulphocyanate administered *per os* reappeared in the urine; that is, the normal animal excreted more than the animal on alcohol, which is just the reverse of what occurs after the administration of acetonitrile.

The above experiments show that there is practically no difference between the amounts of sulphocyanate excreted by normal and alcoholic guinea pigs after the administration of sodium sulphocyanate; hence, the increased excretion by the guinea pigs on alcohol of sulphocyanate, after the administration of acetonitrile, most probably depends upon an increased formation of sulphocyanate, and hence upon an increased breaking up of the molecule of acetonitrile. Whether this increased breaking up of the acetonitrile is due to processes of oxidation (as I suppose to be the case) or to simple cleavage, it is impossible to state. In any case, the increased susceptibility of the alcohol animals is evidently connected with profound modifications of metabolic processes, and thus one case of increased susceptibility can be taken from the vague class of "lowered resistance" and a rational explanation offered.

While it may not be altogether justifiable to bring in Ehrlich's side-chain theory of immunity in this connection, yet this theory enables us to form a mental picture of how the tolerance for alcohol and the increased power to oxidize the methyl group of acetonitrile

might be brought about. If we suppose that alcohol is normally oxidized by reactive chemical groups analogous to side chains, then the tolerance may be thought of as due to an increase in the number of these groups and it is very natural to suppose that an organism that has acquired increased power of oxidizing the ethyl group of alcohol has also acquired increased power of oxidizing the methyl group of acetonitrile. This is but a suggestion; yet it should be remembered that Ehrlich proposed his side-chain theory of the structure of living matter in connection with his work on oxidation and sometime before he began his work on immunity.[a]

This explanation is very similar to the one recently suggested by Vaughan[b] for the "Rosenau-Anderson"[c] or "Theobold Smith"[d] phenomenon (the hypersusceptibility of guinea pigs to horse serum after a previous injection of horse serum). Vaughan supposes the proteid molecules (of horse serum, for example) to contain toxic groups; the first time the horse serum is injected the body breaks up the molecule but slowly, so that the toxic groups are set free slowly, and there are no symptoms of intoxication. But if a second injection is made after ten days "the cells tear the molecule to pieces quicker than before; this sets the poisons free quicker and the animal dies."

These experiments with alcohol and acetonitrile are of interest in another connection. The greatest advance in recent years in our knowledge of the physiological action of alcohol has been the clear demonstration that alcohol is oxidized in the body and may replace fats and carbohydrates and, to a certain extent, the proteids of an ordinary diet. So clear has been this demonstration that the view that alcohol, in moderate amounts, should be regarded as a food is almost universally accepted by physiologists, and the drift of opinion is certainly toward the view that it is in all respects strictly analogous to sugar and fats, provided always that the amount used does not exceed that easily oxidized by the body. Under these premises it would be expected that alcohol in a diet would have the same effect upon an animal's susceptibility to acetonitrile as has dextrose, for example. This is by no means the case, however; on the contrary, the action of these substances in this regard is entirely different. Mice fed upon oats soaked in a solution of dextrose or upon cakes containing considerable dextrose, or upon rice, show a very distinct increase in their resistance to acetonitrile; such mice

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[a] Ehrlich, Das Sauerstoff-Bedürfniss des Organismus, 1885.

[b] Journ. Amer. Med. Assoc., v. 47, p. 1009; 1906.

[c] Bulletin No. 29, Hygienic Laboratory, 1906.

[d] Otto, V. Leuthold-Festschrift, 1906.

may recover from two or even three times the dose fatal to controls.[a] While these facts are not sufficient to justify the conclusion that in many cases alcohol has not a true food value, yet they are sufficient to indicate caution in applying, without further consideration, the brilliant and very exact results on the proteid sparing power of alcohol to practical dietaries.[b]

The method of experimenting pursued in the above, namely, the determination of the effect of the long-continued use of a drug upon the action of other drugs seems adapted to the study of other problems in pharmacology such as those dealing with effects of food preservatives, for example, where experiments made upon healthy men and animals with small doses have often led to as inconclusive results as similar experiments with alcohol.

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[a] It is probable that the explanation of the increased resistance to acetonitrile of animals fed largely upon carbohydrates is that such animals do not break up so much of the nitrile. Thus in experiments upon two dogs, one of which had been kept for some time upon a diet consisting largely of lean meat, the other upon a diet consisting largely of rice, lard, and sugar, the meat-fed dog excreted 1.8 times as much cyanogen in the form of sulphocyanate, after a given dose of acetonitrile, as did the carbohydrate-fed dog; the symptoms of poisoning were also more severe in the former. Proteids are generally held to increase certain physiological oxidations; hence the above results are in accord with the hypothesis that processes of oxidation are involved in poisoning by acetonitrile. The markedly increased resistance of animals receiving a limited amount of food (see experiments on mice above) may similarly be supposed to be due to a lowering of certain of the processes of oxidation in the body; in two experiments on guinea pigs which had received, for some time, a limited diet there was a decidedly smaller excretion of sulphocyanate after the administration of acetonitrile than in the normal animals. Upon such a diet the body seems to acquire the ability to limit the decompositon of this poison just as it does that of the consumption of proteids and energy in certain diseases (Krehl, Clinical Pathology, translation, p. 319). On the other hand it may be, as Mr. Clowes has suggested, that in the case of the large proteid diet the cyanogen, rather than the methyl group, should be considered; that is, that the organism accustomed to dealing with the nitrogen groups of proteids would attack the cyanogen group first, setting it free much more rapidly than it could be neutralized by sulphur.

[b] Chittenden (Med. News, v. 86, p. 721) also cautions against accepting, without reservation, the view that alcohol should be considered a food comparable with the carbohydrates; he bases this view upon Beebe's work on the effect of alcohol upon uric acid excretion which he interprets as showing that alcohol diminishes the oxidation of uric acid. Chittenden expresses his conclusions in the following forceful words: "However this may be, it is, I think, quite plain that while alcohol in moderate amounts can be burned in the body, thus serving as food in the sense that it may be a source of energy, it is quite misleading to attempt a classification or even comparison of alcohol with carbohydrates and fats, since, unlike the latter, alcohol has a most disturbing effect upon the metabolism or oxidation of the purin compounds of our daily food. Alcohol, therefore, presents a dangerous side wholly wanting in carbohydrates and fats. The latter are simply burned up to carbonic acid and water, or are transformed into glycogen and fat, but alcohol, though more easily oxidizable, is at all times liable to obstruct, in some measure at least, the oxidative processes of the liver, and probably of other tissues also, thereby throwing into the circulation bodies such as uric acid which are inimical to health; a fact which at once tends to draw a distinct line of demarcation between alcohol and the two nonnitrogenous foods—fat and carbohydrate."

II.

THE EFFECT OF ALCOHOL UPON THE SULPHUR OF THE URINE.

1. *Effect of alcohol upon the excretion of ethereal sulphates.*—The following experiments, although very incomplete, seem worth recording, for they bring out an action of alcohol which has apparently been but seldom noticed. Briefly stated, it is shown that the excretion of ethereal sulphates in the urine may be increased many times, both absolutely and relatively to the inorganic sulphates, by the administration, continued for some time, of alcohol. It was found, for example, that their absolute amount might be increased in the rabbit from 8 or 9 milligrams per day to over 100 milligrams, while the percentage of sulphuric acid excreted in this form might increase from 3 or 4 to 50.

There are a few statements in the literature on alcohol which might have suggested that such a result would be found. Edsall,[a] for example, found the urine in a number of cases of chronic alcoholism to contain large amounts of phenol, a substance usually excreted in combination with sulphuric acid; he was inclined to regard this as an indication that the liver had been injured by alcohol and so was unable to destroy as much phenol as normally.[b] De Schweinitz and Edsall[c] reported a number of cases of tobacco-alcohol amblyopia in which there were present in the urine abnormal amounts of indican, phenol, and of total ethereal sulphates; they concluded that toxic substances produced in the digestive tract probably have a part in the production of this form of amblyopia.

Herter in his Lectures on Chemical Pathology (p. 161) suggested that the gastritis[d] and motor disturbances following the abuse of alcohol may lead to increased putrefaction and that the products of the latter inflict injuries to the liver which may ultimately be a factor in the production of cirrhosis of the liver.

Experimental.[e]—The experiments were performed upon rabbits. The alcohol was introduced into the stomach by means of a soft rubber

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[a] Univ. of Penn. Med. Bull., v. 16, p. 436; 1903–4.

[b] That narcotic drugs may inhibit the ability of the liver to transform phenol was shown by Herter and Wakeman (Jour. Exper. Med., v. 4, p. 322; 1899), who found that less phenol could be recovered, by distillation, from the liver of a normal animal than from one which had been anæsthetized for a long time.

[c] Amer. Jour. Med. Sci., v. 126, p. 216; 1903.

[d] Jagie (Wien. klin. Woch., v. 19, p. 1058; 1906) found alcoholic gastritis and enteritis as the initial symptoms of cirrhosis of the liver in a large number of cases.

[e] A number of the earlier sulphate determinations in these experiments were made by Mr. M. B. Porch, formerly assistant in pharmacology, to whom I wish to express my thanks.

catheter; it was always warmed to the body temperature and diluted with water to about 25 per cent. The same food (cabbage and carrots) was given throughout the entire experiment; no attempt was made to determine the amount eaten. The urine was collected twice a day from a dish placed under the cage; the amount collected in twenty-four hours varied considerably. The sulphur determinations were made by Folin's method,[a] Gooch crucibles being used. Duplicate analyses were always made.

EXPERIMENT I.—*Rabbit, dark gray; alcohol daily after January 15.*

Date.	Weight in grams.	Alcohol.		Total S as SO_3 in milligrams.	Total sulphates as SO_3 in milligrams.	Inorganic sulphates as SO_3 in milligrams.	Ethereal sulphates as SO_3 in milligrams.	Percentage of sulphates as ethereal sulphates.
		In grams.	Grams per kilo.					
1905.								
Dec. 14	2,400	.....	.....	.....	.....	.....	.....	.....
Dec. 21	2,400	.....	.....	.....	.....	.....	.....	.....
Dec. 25	2,390	.....	.....	.....	.....	.....	.....	.....
1906.								
Jan. 2	2,320	.....	.....	.....	.....	.....	.....	.....
Jan. 8–9	.....	.....	.....	442.5	[a] 279.2	269.9	9.3	3.3
Jan. 9–11	.....	.....	.....	.....	[b] 266.9	258.8	8.1	3.0
Jan. 15	2,270	6.81	3	.....	.....	.....	.....	.....
Jan. 21–22	.....	.....	.....	.....	59.7	34.0	25.7	43.0
Jan. 22–23	.....	.....	.....	.....	89.1	64.9	24.2	27.0
Jan. 24	2,070	.....	.....	.....	.....	.....	.....	.....
Jan. 26	2,040	.....	.....	.....	.....	.....	.....	.....
Feb. 7–8	.....	.....	.....	.....	189.7	175.9	13.8	7.3
Feb. 8–9	.....	.....	.....	.....	115.0	106.0	9.0	7.8
Feb. 9	2,090	.....	.....	.....	.....	.....	.....	.....

[a] 64.5 per cent of total S. [b] Mean of two days.

Thus, within a week after beginning the alcohol the absolute amount of the total sulphate had decreased markedly, but the ethereal sulphates had increased from 8 or 9 milligrams to about 25 milligrams; the percentage of sulphate excreted as ethereal sulphate had increased from about 3 to 43. The same dose of alcohol (3 grams per kilo) was continued daily (with the occasional intermission of a day) for three weeks; both the absolute and relative amount of ethereal sulphate fell. This effect may have been due to the establishment of tolerance for the alcohol.

[a] Jour. Biol. Chem., v. 1, p. 131; 1906. In working with rabbits' urine I have found it necessary to frequently renew the asbestos mats of the Gooch crucibles; otherwise they either allowed a little barium sulphate to pass through or the filtration, even with strong pressure, became extremely slow. I have found the method to work admirably with normal human and dog's urine.

EXPERIMENT II.—*Rabbit, black; alcohol daily after November 4.*

Date.	Weight in grams.	Alcohol.		Total S as SO_3 in milligrams.	Total sulphates as SO_3 in milligrams.	Inorganic sulphates as SO_3 in milligrams.	Ethereal sulphates as SO_3 in milligrams.	Percentage of sulphates as ethereal sulphates.
		In grams.	Grams per kilo.					
1905.								
Nov. 2–3	2,035	.....	.....	.....	260.7	248.2	12.5	4.8
Nov. 3–4	.....	.....	.....	.....	281.6	270.6	11	3.9
Nov. 4	2,020	4.04	2	.....	.....	.....	.....	.....
Nov. 13	2,075	.....	.....	.....	.....	.....	.....	.....
Nov. 23	2,120	.....	.....	.....	.....	.....	.....	.....
Nov. 28	2,230	6.7	3	.....	.....	.....	.....	.....
Dec. 7	[a] 2,350	9.4	4	.....	.....	.....	.....	.....
Dec. 9	.....	8.2	3.5	.....	.....	.....	.....	.....
Dec. 18	2,190	.....	.....	.....	.....	.....	.....	.....
1906.								
Jan. 8–9	.....	.....	.....	346	[b] 224.4	194.9	29.5	13.1
Jan. 9–10	.....	.....	.....	365.2	[c] 239	212.8	26.2	10.9

[a] Had 6 young December 11. [b] 64.8 per cent of total S. [c] 65.4 per cent of total S.

In the following experiment there was a return of the ethereal sulphate excretion to normal when the alcohol was discontinued.

EXPERIMENT III.—*White rabibt; alcohol daily from October 8 to December 7.*

Date.	Weight in grams.	Alcohol.		Total sulphates as SO_3 in milligrams.	Inorganic sulphates as SO_3 in milligrams.	Ethereal sulphates as SO_3 in milligrams.	Per centage of sulphates as ethereal sulphates.
		In grams.	Grams per kilo.				
1906.							
Oct. 5–6	2,140	.....	.....	327.8	315.6	12.2	3.7
Oct. 6–7	.....	.....	.....	225.2	210	15.2	6.8
Oct. 8	2,150	4.3	2	.....	.....	.....	.....
Oct. 10	2,060	.....	.....	.....	.....	.....	.....
Oct. 15	2,140	.....	.....	.....	.....	.....	.....
Oct. 16–17	.....	.....	.....	372.8	354.7	18.1	4.9
Oct. 18	2,030	.....	.....	.....	.....	.....	.....
Oct. 19	2,030	6.09	3	.....	.....	.....	.....
Oct. 20	2,020	.....	.....	.....	.....	.....	.....
Oct. 23–24	2,040	.....	.....	314	308.6	5.4	1.7
Oct. 25	2,070	.....	.....	.....	.....	.....	.....
Oct. 30	2,010	.....	.....	.....	.....	.....	.....
Nov. 5	2,010	.....	.....	.....	.....	.....	.....
Nov. 6	2,010	7.04	3.5	.....	.....	.....	.....
Nov. 10	1,985	.....	.....	.....	.....	.....	.....
Nov. 15	2,020	.....	.....	.....	.....	.....	.....
Nov. 15–17	.....	.....	.....	[a] 194.8	145.5	49.3	25.3
Nov. 17–19	.....	.....	.....	[a] 165.3	130.5	34.8	21.1
Nov. 19	2,020	8.08	4	.....	.....	.....	.....
Nov. 22	1,990	8.96	4.5	.....	.....	.....	.....
Nov. 25	1,950	.....	.....	.....	.....	.....	.....
Nov. 26–7	.....	.....	.....	180	135.6	44.4	24.7
Nov. 27–28	.....	.....	.....	180	125.3	54.7	30.4
Nov. 30	1,935	.....	.....	.....	.....	.....	.....
Dec. 4–6	.....	.....	.....	[a] 200.6	96.7	103.9	51.8
Dec. 6	1,920	.....	.....	.....	.....	.....	.....
Dec. 7	(b)	(b)	(b)	(b)	(b)	(b)	(b)
Dec. 15	2,030	.....	.....	.....	.....	.....	.....
Dec. 15–17	.....	.....	.....	[a] 186	182.3	3.7	1.9

[a] Mean. [b] Alcohol discontinued.

The rabbit used for the following experiment was very resistant to alcohol, and the characteristic urinary changes occurred only after the administration of large doses.

EXPERIMENT IV.—*Rabbit, brown; alcohol daily from October 10.*

Date.	Weight in grams.	Alcohol.		Total sulphates as SO_3 in milligrams.	Inorganic sulphates as SO_3 in milligrams.	Ethereal sulphates as SO_3 in milligrams.	Per centage of sulphates as ethereal sulphates.
		In grams.	Grams per kilo.				
1906.							
Oct. 5	2,310	.....	.....	.....	.....	.....	.....
Oct. 7–8	.....	.....	.....	296.9	287.1	9.8	3.3
Oct. 8–9	.....	.....	.....	180.2	175	5.2	2.9
Oct. 10	2,220	4.44	2	.....	.....	.....	.....
Oct. 15	2,180	.....	.....	.....	.....	.....	.....
Oct. 18–19	.....	.....	.....	275.7	266.8	8.9	3.2
Oct. 20	2,190	6.57	3	.....	.....	.....	.....
Oct. 25	2,240	.....	.....	.....	.....	.....	.....
Oct. 30	2,280	.....	.....	.....	.....	.....	.....
Oct. 31 / Nov. 1	.....	.....	.....	438.6	424.8	13.8	3.1
Nov. 5	2,310	.....	.....	.....	.....	.....	.....
Nov. 6	2,310	8.05	3.5	.....	.....	.....	.....
Nov. 10	2,350	.....	.....	246	212.9	33.1	13.5
Nov. 15	2,140	.....	.....	.....	.....	.....	.....
Nov. 16	2,140	8.56	4	.....	.....	.....	.....
Nov. 19–20	.....	.....	.....	194.8	159.1	35.7	18.3
Nov. 22	2,020	9.09	4.5	.....	.....	.....	.....
Nov. 25	2,270	.....	.....	.....	.....	.....	.....
Nov. 26	2,270	11.35	5	.....	.....	.....	.....
Nov. 30	2,135	.....	.....	.....	.....	.....	.....
Dec. 1–2	.....	.....	.....	243.2	215.7	27.5	11.3
Dec. 2–3	.....	.....	.....	214.	188.1	25.9	12.1
Dec. 5	2,170	11.94	5.5	.....	.....	.....	.....
Dec. 10	2,100	.....	.....	.....	.....	.....	.....
Dec. 13	2,140	12.84	6	.....	.....	.....	.....
Dec. 15	2,150	.....	.....	.....	.....	.....	.....
Dec. 18	2,220	14.43	6.5	.....	.....	.....	.....
Dec. 20	2,150	.....	.....	.....	.....	.....	.....
Dec. 20–21	.....	.....	.....	175.1	124.1	51	29.1
Dec. 22	2,160	15.12	7	.....	.....	.....	.....
Dec. 27	2,090	.....	.....	.....	.....	.....	.....
Dec. 31	2,030	.....	.....	.....	.....	.....	.....
1907.							
Jan. 3	1,880	.....	.....	.....	.....	.....	.....
Jan. 4–5	.....	.....	.....	[b] 58.2	42.5	15.7	27.0

[a] Died. [b] From urine for about ten hours before death.

Sulphate determinations were frequently made upon the urine of normal rabbits kept under the same conditions as those which were receiving alcohol, the object being to determine if prolonged confinement would cause any increase in the excretion of ethereal sulphates. The latter did not occur. There were some variations in both the absolute amount of ethereal sulphates and in the percentage, but these never reached the figures constantly obtained with rabbits receiving alcohol.

Results similar to the above were obtained in two experiments upon guinea pigs. Under the influence of alcohol the percentage of the ethereal sulphates increased from 1 or 2 to 30 or 37.

Discussion.—The most striking effect of alcohol shown in the above tables is the increase in the excretion of the ethereal sulphates.[a] We may now take up the question as to the probable cause of this increase.

The views as to the significance of the ethereal sulphates are not in entire accord. For some time after their discovery by Baumann they were generally held to be an index of the amount of intestinal putrefaction. The correctness of this interpretation was, however, sometimes questioned. Thus Schütz,[b] in 1901, called attention to the fact that a variable amount of these products are excreted in the feces and that another part is destroyed in the organism; hence but a part of those formed appear in the urine.

Very recently Folin[c] and Shaffer[d] have reported experiments tending to show that not all of the ethereal sulphate present in the urine comes from the absorption of the products of intestinal putrefaction. Thus Folin found the indican (one of the most prominent of the ethereal sulphates) to disappear from the urine entirely upon a starch and cream diet, while the absolute quantity of ethereal sulphates was reduced only to about one-half of the amount eliminated on a nitrogen-rich diet. Folin concludes from this and other observations: "The ethereal sulphates can only in part be due to intestinal putrefaction, and neither their absolute nor their relative amount can be accepted as an index of the extent to which putrefaction is taking place in the intestines." Folin drew his conclusions from observations upon normal individuals. While they seem to show clearly that there is normally excreted some ethereal sulphate which is not connected with intestinal putrefaction, yet there seems to be nothing in them contrary to the view that when the ethereal sulphates are largely increased in pathological conditions this increase is not due chiefly to increased intestinal putrefaction. It may be added that v. Tabora[e] and Koziczkowsky,[f] two of the latest writers on this

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[a] There was in nearly all cases a diminution in the total sulphate excretion. As the sulphate excretion is, generally speaking, parallel to the proteid katabolism, the above results indicate that a smaller amount of proteid was being katabolized under the influence of alcohol. In the absence of data concerning the intake of food, it is not possible to determine whether this result was due to the proteid-sparing power of the alcohol or to the animals eating less.

[b] Arch. f. Verdauungskrank., v. 7, p. 43.

[c] Amer. Journ. Physiol., v. 13, p. 99, 1905.

[d] Ibid., v. 17, p. 380, 1906.

[e] Dtsch. Arch. f. klin. Med., v. 87, p. 254, 1906.

[f] Zeit. f. klin. Med., v. 57, p. 413.

subject, found a parallelism, although not complete, between the excretion of total ethereal sulphates and of indican.

Pringsheim[a] has recently made a suggestion which is of special interest in this connection, namely, that after the administration of alcohol some of this is excreted as the ethyl ether of sulphuric acid.[b] His arguments and experiments, are, however, not at all conclusive. This is a point well worth further investigation, however.

Accepting what seems to be the most probable explanation of the great increase in the ethereal sulphates in the above experiments, namely, that they have their origin in increased intestinal putrefaction, we may now consider how such increased putrefaction may be caused by alcohol. Among the numerous conditions to which increased intestinal putrefaction has been attributed there are two of special interest in this connection—chronic intestinal catarrh and diminution of free hydrochloric acid in the stomach. Both of these are well-known results of the administration of alcohol. Thus, Friedenwald found a gradual reduction in free hydrochloric acid in the gastric contents of his experimental animals. The relation of the hydrochloric acid of the gastric juice to intestinal putrefaction has, however, been the subject of much discussion. The view of Kast, Wasbutzki, and Stadelmann that absence or diminution of hydrochloric acid in the gastric juice led to increased intestinal putrefaction was opposed by von Noorden, but was again accepted by Biernacki, Schmitz, and others. Schultz[c] in 1901 again brought up arguments against Kast's view; these have recently been criticised by v. Tabora,[d] who, from a number of careful experiments, concludes that the hydrochloric acid prevents to a certain degree intestinal putrefaction and that subacidity and anacidity as a rule favor it.

Assuming that the great increase in the excretion of ethereal sulphate in these experiments is to be interpreted as showing that alcohol leads to increased intestinal putrefaction, the question arises, Can any of the pathological effects of alcohol be ascribed to this; in other words, may some tissues or organs be injured by the products of this intestinal putrefaction? Attention has already been called to the work of de Schweinitz and Edsall in regard to the relation of the products of intestinal putrefaction to tobacco-alcohol amblyopia. Elschnig[e] has reported a series of cases in which there seemed to

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[a] Zeit. f. physikal. u. diät. Therapie, v. 10, p. 281; 1906.

[b] If this ether were formed it would doubtless be excreted unchanged in the urine, for Salkowski (Virchow's Arch., v. 66, p. 315) found that it undergoes no change in the body.

[c] Arch. f. Verdauungskrank., v. 7, p. 43.

[d] Deutsch. Arch. f. klin. Med., v. 87, p. 254; 1906.

[e] Elschnig, Münch. med. Woch., 52, No. 41, Oct. 10, 1905; Klin. Monatsh. f. Augenheilk., v. 43, p. 417; 1905.

be a parallelism between the amount of indican in the urine and various more or less severe eye disturbances. This entire subject has recently been discussed by de Schweinitz and others.[a] Certain other symptoms, such as some forms of headaches and neurasthenia, have, apparently with good reason, been attributed to these products; nephritis following intestinal obstruction has likewise been ascribed to the products of intestinal putrefaction.

The most interesting suggestion as to a possible pathological significance of the products of intestinal putrefaction is that they may have a part in the causation of cirrhosis of the liver. The view has often been expressed that cirrhosis of the liver is dependent in some way upon autointoxication from the digestive tract. Krawkow claimed to have obtained cirrhosis of the liver in fowls by the feeding of an infusion of putrid horse meat. One of the most definite suggestions in this connection is that of Boix,[b] who believed that the fatty acids formed as a result of gastritis were a factor in the causation of cirrhosis; he claimed to have obtained cirrhotic changes by the administration of butyric and acetic acids to animals.[c] There is, at present, no ground for supposing that those products (indol, skatol, phenol, etc.), which are usually considered the typical products of intestinal putrefaction, and which are largely responsible for the ethereal sulphates in the urine, have much significance in this respect; some of the most notable failures to produce cirrhosis of the liver experimentally have been in experiments upon rabbits; that is, upon animals in which alcohol readily leads to a great increase in the excretion of ethereal sulphates. This by no means excludes the possibility, however, that in some cases such products may contribute to such a result. Furthermore, evidence is gradually accumulating that indol, for example, which has ordinarily a very low degree of toxicity, may, under certain conditions, become distinctly toxic. Thus, Richards and Howland[d] found it decidedly toxic to animals whose powers of oxidation are lowered by potassium cyanide; similar results were obtained with phenol.

Recent experiments of Porcher and Hervieux[e] suggest another way in which the toxicity of indol may possibly become enhanced. These authors find indol () to be but very slightly toxic, while comparatively small amounts of indoxyl (), injected subcutane-

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[a] Jour. Amer. Med. Assoc., v. 48, pp. 502, 543; 1907.

[b] Le foie des dyspéptiques, Paris Thesis, 1895.

[c] These conclusions have been recently unfavorably criticized by Goannoviecs (Arch. int., de Pharmacodynamie, v. 15, p. 241; 1905).

[d] Proc. Soc. Exper. Biol. & Med., v. 3, p. 71; 1905–6; cf. Herter, Medical Record, v. 70, p. 788; 1906.

[e] Jour. de Phys. et de Path. gén., v. 18, p. 841; 1906.

ously, are rapidly fatal. As is well known, indol, whether it be administered or be formed in the intestine, is normally excreted as indoxyl-sulphuric acid, which is also practically nontoxic. In other words, indol, before it can be excreted, is converted into the poisonous compound indoxyl. The latter is probably normally conjugated at once with sulphuric acid (or if present in large amounts converted into harmless precursors of indigo—not, according to these authors, into glycuronates), but it is conceivable that pathological conditions may arise which prevent this, and that then the indoxyl may have a deleterious effect.[a]

A systematic study of the excretion of ethereal sulphates, phenol, etc., in cases of alcoholism in man would probably yield interesting results. In a single observation upon a man with advanced alcoholic cirrhosis of the liver the urine contained 4.2 per cent of ethereal sulphates, which is lower than what is usually considered normal; unfortunately, data concerning the diet and the absolute amount of sulphates excreted were not available. It would have been interesting to have had phenol and indoxyl determinations in this case; it is possible that the liver had lost the power of neutralizing these poisons with sulphuric acid.[b]

2. *Effect of alcohol upon the neutral sulphur of the urine.*—As is well known, sulphur is constantly present in the urine in forms other than sulphuric acid. This is known as neutral or unoxidized sulphur and is made up of a very miscellaneous group of substances (sulphocyanates, cystin, taurin-derivatives, chondroitic sulphuric acid, oxy- and alloxyproteic acids, etc.). The taurin of the bile has long been quoted as an important source of the neutral sulphur. This view was based largely upon the work of Kunkel.[c] Recent experiments by Shaffer[d] throw much doubt upon the correctness of this conclusion, at least as far as man is concerned.[e] The elimination of neutral sulphur is not so closely dependent upon the sulphur of the food as is that of the sulphates.[f]

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[a] A suggestive illustration of the cumulative effects of two substances may be found in the relation of a large meat and alcohol diet to gouty manifestations. According to interesting observations made in Italy, neither the consumption of meat alone nor of alcohol with a low meat diet has a special tendency to lead to gouty attacks. Such a result, however, occurs frequently with a diet containing both. (Wood, Therapeutics, 12th ed., p. 308; 1905.)

[b] Edsall (l. c. and Boston Med. and Surg. Jour., v. 156, p. 181; 1907) believes that the increase in the ethereal sulphates, which is usually interpreted as indicating increased intestinal putrefaction, may be due, in part, to abnormalities in the liver, excretory, or various other organs.

[c] Pflüger's Archiv., v. 14, p. 344; 1877.

[d] Amer. Journ. Physiol., v. 17, p. 374; 1906.

[e] It is interesting to note in this connection that Kunkel did not include the ethereal sulphates with the total sulphates.

[f] Folin, Amer. Journ. Physiol., v. 13, p. 99, 1905; cf. Shaffer, l. c.

In my experiments, alcohol seemed to have but little effect upon the percentage of sulphur excreted as neutral sulphur. Thus, in Experiment I (see above), the neutral sulphur amounted to 35.5 per cent of the total sulphur excreted by a normal rabbit. In Experiment II, after the animal had received alcohol for over two months (the diet being otherwise the same), and there had been a marked increase in the excretion of ethereal sulphates, the neutral sulphur constituted approximately 35 per cent of the total sulphur. Two other experiments gave similar results; the percentage of sulphur excreted as neutral sulphur varied in the normal rabbit from 32.3 to 34.3, and in the rabbit receiving alcohol from 30.4 to 31.3 per cent.

In a case of advanced alcoholic cirrhosis of the liver (man) 7.3 per cent of neutral sulphur was found; this figure is very similar to those obtained by Folin and Shaffer for men on a high proteid diet. Unfortunately no data as to the diet in this case, which is of such importance, are available.

An increased excretion of neutral sulphur is frequently interpreted as indicating a diminution of physiological oxidation. Some of the arguments for this view are furnished by the work of Reale and Boeri,[a] who found the neutral sulphur of the urine increased when the respiration was interfered with mechanically, and by the work of Harnack and Kleine[b] on the effects of alkalis and chloral hydrate on the excretion of neutral sulphur. The results of my experiments with acetonitrile are probably to be similarly interpreted; in poisoning by this substance the percentage of neutral sulphur frequently rose from 25 to 65 or more; the total sulphur excretion was not much changed but the oxidized sulphur frequently almost disappeared. Part of the increased neutral sulphur was contained in the sulphocyanate formed from the nitrile, but the great decrease in the sulphates suggests that physiological oxidations had been lowered by the nitrile. Returning to the experiments with alcohol: This substance did not cause an increase in the excretion of neutral sulphur; this may be considered as another argument that alcohol has but a limited power of inhibiting "physiological oxidations."[c]

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[a] Reale and Boeri, Wien. med. Woch., 1895, p. 1198.

[b] Harnack and Kleine, Zeit. f. Biol., v. 37, p. 417; Kleine, Inaug. Diss., Halle; 1895.

[c] Inasmuch as no one seems to have recorded sulphur determinations in the urine of guinea pigs it may be of interest to note that these animals, receiving a diet consisting largely of carrots and cabbage (as did the rabbits) excreted from 25 to 35 per cent of the sulphur as neutral sulphur. This ratio was not changed in animals inoculated with tubercle bacilli.

Kittens receiving a diet of bread and milk excreted from 25 to 30 per cent of the sulphur as neutral sulphur. A dog receiving a diet consisting almost entirely of lean meat excreted from 23 to 29 per cent of the sulphur as neutral sulphur; one on a diet consisting largely of rice, lard, and sugar excreted from 64 to 65 per cent in this form.

In a case of acute catarrhal jaundice (man) 15 per cent of the sulphur was in the neutral form; this figure is lower than that frequently found, but in the absence of data as to diet the figures have not much value. There was 16.4 per cent of ethereal sulphates in this case.